201-15352B

Robust Test Summaries for 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-γ-2-benzopyran (HHCB) CAS# 1222-05-5

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Submitted to the EPA under the HPV Challenge Program by:

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Robust Summary for HHCB

The evaluation of the quality of the following data uses a systematic approach described by Klimisch [Klimisch *et al*., 1996]. Based on criteria relating to international testing standards for categorizing data reliability, four reliability categories have been established. The following categories are:

- Reliability code 1. Reliable without restrictions
- Reliability code 2. Reliable with restrictions
- Reliability code 3. Not reliable
- Reliability code 4. Not assignable

1 Chemical and Physical Properties

1.1 Melting Point

Substance Name	HHCB
CAS No.	1222-05-5
Method/guideline	Sample was cooled to –30° C and gradually warmed
GLP	No
Melting Point	-10 to 0 degrees C
Data Qualities Reliabilities	Reliability 2. Reliable with restriction
Remarks for Data Reliability	The substance is a mixture of isomers and is not expected to have a precise melting point.
References	IFF, 2001

1.2 Boiling Point

Substance Name	HHCB
CAS No.	1222-05-5
Method/guideline	Calculated using Syracuse Research Corporation SAR-based software
GLP	No
Year	2000
Boiling Point	162° C @ 760 mm
Data Qualities Reliabilities	Reliability 4. Not assignable
Remarks for Data Reliability	Data calculated by recognized SAR program with input of log Kow, VP and water solubility.
References	William Meylan and Philip Howard, 2000. EPI Suite v 3.10, Syracuse Research Corporation

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Substance Name	HHCB
CAS No.	1222-05-5
Method/guideline	Measured during distillation
GLP	No
Year	2001
Boiling Point	160° C @ 4 mm Hg
Data Qualities Reliabilities	Reliability 2. Reliable with restriction
Remarks for Data Reliability	The measured boiling point was recorded in the distillation of HHCB in the manufacturing plant.
References	IFF, 2001

1.3 Vapor Pressure

Substance Name	HHCB
CAS No.	1222-05-5
Method/guideline	OECD 104
Vapor Pressure	0.0727 Pa
Temperature	25° C
Data Qualities Reliabilities	Reliability 1. Reliable without restrictions
Remarks for Data Reliability	Study conducted according to an OECD protocol under GLP and data are published in a peer-reviewed journal
References	Balk F and Ford RA, 1999a. Environmental risk assessment for the polycyclic musks AHTN and HHCB in the EU. I. Fate and Exposure Assessment. Toxicology Letters, 111, 57-79.

1.4 n-Octanol/Water Partition Coefficient

Substance Name	HHCB
CAS No.	1222-05-5
Method/guideline	OECD 117
GLP	Yes
Year	1999
Log Pow	5.9
Temperature	25° C
Remarks for Test Conditions	Conducted under GLP
Remarks for Results	Result is an average of 5.8 and 6.0, the values for the 2 principal isomers
Data Qualities Reliabilities	Reliability 1. Reliable without restrictions
Remarks for Data Reliability	Study conducted according to an OECD protocol under GLP and data are published in a peer-reviewed journal.
References	Balk F and Ford RA, 1999a. Environmental risk assessment for the polycyclic musks AHTN and HHCB in the EU. I. Fate and Exposure Assessment. Toxicology Letters, 111, 57-79.

1.5 Water Solubility

Substance Name	HHCB
CAS No.	1222-05-5
Method/Guideline	OECD 105
GLP	Yes
Year	1996
Value (mg/L) at Temperature	1.75 @ 25° C at a pH of 7
Remarks for Test Conditions	
Remarks for Results	Water solubility was 1.99 mg/L at a pH of 5 and 1.69 mg/L at a pH of 9.
Data Qualities Reliabilities	Reliability 1. Reliable without restrictions
Remarks for Data Reliability	Study conducted according to an OECD protocol under GLP and data are published in a peer-reviewed journal
References	Balk F and Ford RA, 1999a. Environmental risk assessment for the polycyclic musks AHTN and HHCB in the EU. I. Fate and Exposure Assessment. Toxicology Letters, 111, 57-79.

1.6 Relative Density

Substance Name	HHCB
CAS No.	1222-05-5
Method/Guideline	OECD 109
GLP	No
Year	2001
Value (mg/L) at Temperature	0.99 – 1.015 g/cm^3 @ 20° C
Remarks for Test Conditions	Measured with an oscillating densitometer.
Remarks for Results	
Data Qualities Reliabilities	Reliability 2. Reliable with restrictions
Remarks for Data Reliability	

References	IFF, 2001

1.7 Flashpoint

Substance Name	HHCB
CAS No.	1222-05-5
Method/Guideline	Pensky Martens Method (closed cup)
GLP	No
Year	2001
Value (mg/L) at Temperature	>100° C
Remarks for Test Conditions	
Remarks for Results	
Data Qualities Reliabilities	Reliability 2. Reliable with restrictions
Remarks for Data Reliability	
References	IFF, 2001

2 Environmental Fate and Pathways

2.1 Photodegradation

Substance Name	HHCB
CAS No.	1222-05-5
Method/guideline	
Test Type	Irradiation
Half-life t1/2	3.7 hours
Remarks for Test Conditions	Black irradiation lamps of λ >300 nm at 25° C and 740 mmHg.
Remarks for Results	
Data Qualities Reliabilities	Reliability 1. Data are reliable without restriction
Remarks for Data Reliability	Data obtained under laboratory conditions using methyl vinyl ketone as a reference substance.
References	Aschman SM, Arey J, Atkinson R and Simonich SL, 2001. Atmospheric lifetimes and fates of selected fragrance materials and volatile model compounds. Environmental Science and Technology, 359180, 3595-3600.

2.2 Stability in Water

Substance Name	HHCB
CAS No.	1222-05-5
Method/guideline	
Test Type	
Remarks for Test Conditions	
Remarks for Results	No physical test was conducted to prove that HHCB is stable in water. Based on the chemical structure, HHCB is predicted and expected to be unreactive with water. The potential for any significant hydrolysis reaction at the benzylic position is minimized. In order to facilitate hydrolysis of the benzyl ether moiety, either a high concentration of strong aqueous acids or a large quantity of metal salts would be required. Additionally, HHCB has low water solubility, thereby reducing the potential for reaction even further. Thus, there is no significant potential for the hydrolysis of HHCB in water.

Data Qualities Reliabilities	Reliability 4. Not assignable
Remarks for Data Reliability	
References	This is the “no test” rationale for the stability of HHCB in water.

2.3 Biodegradation

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	HHCB undiluted (Purity 99%)
Method	OECD 301B
Test Type	Ready Biodegradability
GLP	Yes
Year	1994
Contact Time	28 days
Innoculum	Sewage effluent 1drop/L
Remarks for Test Conditions	Modified Sturm, $C0_2$ Evolution, Sodium benzoate as reference substance.
Degradation % After Time	0%
Time required for 10% degradation	
Remarks Results	HHCB is not mineralized in the ready biodegradability test.
Conclusion Remarks	Further tests have shown that HHCB is inherently biodegradable.
Data Qualities Reliabilities	Reliability 1. Data are reliable without restriction
Remarks for Data Reliability	Data generated using approved OECD protocol under GLP and also published in a peer-reviewed journal.
Reference	Balk F and Ford RA, 1999a. Environmental risk assessment for the polycyclic musks AHTN and HHCB in the EU. I. Fate and Exposure Assessment. Toxicology Letters, 111, 57-79.

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Commercial sample (HHCB in diluent isopropyl myristate)
Method	Modified OECD 301B
Test Type	Ready Biodegradability
GLP	Yes
Year	1993
Contact Time	28 days
Innoculum	Sewage effluent from SCAS after 8 weeks adaptation, 1drop/L
Remarks for Test Conditions	Sealed vessel Total Inorganic Carbon (TIC) test, Benzyl alcohol as reference substance.
Degradation % After Time	0% (corrected for isopropyl myristate)
Time required for 10% degradation	
Remarks Results	HHCB is not mineralized in the ready biodegradability test.
Conclusion Remarks	Further tests have shown that HHCB is inherently biodegradable.
Data Qualities Reliabilities	Reliability 1. Data are reliable without restriction
Remarks for Data Reliability	Data generated using approved OECD protocol under GLP and also published in a peer-reviewed journal.
Reference	Balk F and Ford RA, 1999a. Environmental risk assessment for the polycyclic musks AHTN and HHCB in the EU. I. Fate and Exposure Assessment. Toxicology Letters, 111, 57-79.

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Commercial sample (HHCB in diluent)
Method	
Test Type	Primary Degradation
GLP	No
Year	
Contact Time	

Remarks for Test Conditions	64 samples from different soil types were screened for the presence of naturally occurring micro-organisms. Pure cultures of fungi (Aureobasidium pullulans and Phanerochaete chrysosporium) were incubated with HHCB. Ethyl acetate extracts of the cultures were analysed by GC MS.
Conclusion Remarks	HHCB was demonstrated to degrade to more polar metabolites with the lactone and the hydroxycarboxylic acid as likely intermediates.
Data Qualities Reliabilities	Reliability 2. Data are reliable with restrictions
Remarks for Data Reliability	Data published in a peer-reviewed journal.
Reference	Balk F and Ford RA, 1999a. Environmental risk assessment for the polycyclic musks AHTN and HHCB in the EU. I. Fate and Exposure Assessment. Toxicology Letters, 111, 57-79.

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Radiolabeled HHCB
Method	
Test Type	Soil Microcosm primary degradation
GLP	
Year	1998
Contact Time	1 year
Remarks for Test Conditions	Samples were taken from 1) oak forest 2) agricultural field, 3) sediment of the Delaware river in central New Jersey and 4) sludge amended soil from a farm. Sealed flasks with soil spiked with 10 ug HHCB/g soil were incubated at laboratory temperatures for 1 year. Closed systems were used, with periodic flushing of headspace for oxygen replenishment and effluent gas was drawn through a train of scintillation fluids to capture volatiles and CO2. After the incubation period, the flasks were extracted with solvent and analysed for HHCB.
Conclusion Remarks	An average of 14% of HHCB remained in the soil after one year demonstrating a half-life value of 4 months for HHCB in soils.
Data Qualities Reliabilities	Reliability 2. Data are reliable with restriction
Remarks for Data Reliability	Data published in a peer-reviewed journal.
Reference	Balk F and Ford RA, 1999a. Environmental risk assessment for the polycyclic musks AHTN and HHCB in the EU. I. Fate and Exposure Assessment. Toxicology Letters, 111, 57-79.

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Radiolabeled HHCB
Method	
Test Type	Biotransformation
GLP	
Year	2000
Contact Time	
Remarks for Test Conditions	To understand the fate of HHCB in the environment, biotransformation was examined under realistic conditions in activated sludge and river water. Radiolabeled HHCB was dosed to freshly collected activated sludge (25 ug/L) and river water (1 ug/L). The disappearance of parent and the formation of metabolites were monitored over time.
Conclusion Remarks	The half-lives for parent HHCB were 21 hours in activated sludge and 33 hours in river water. HHCB is biotransformed in activated sludge and river water to polar metabolites that are predicted to be less bioaccumulative and less toxic than the parent compound. Therefore, concentrations of HHCB measured in the environment are lower than predicted concentrations.
Data Qualities Reliabilities	Reliability 2. Data are reliable with restriction
Remarks for Data Reliability	Data published in a peer-reviewed journal.
Reference	Balk F and Ford RA, 1999a. Environmental risk assessment for the polycyclic musks AHTN and HHCB in the EU. I. Fate and Exposure Assessment. Toxicology Letters, 111, 57-79. Langworthy DE, Itrich NR, Simonich SL, and Federle TW, 2000. Biotransformation of the Polycyclic Musk HHCB in activated sludge and river water. Presented at SETAC, May 2000, Brighton, U.K.

2.4 Fugacity

Substance Name	HHCB

CAS No.	1222-05-5
Model Conditions	25° C, 100,000 lbs
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EPT V 3.10 Level III
Input Parameters	MW – 258.41, measured log Kow – 5.9 at 25 degrees C, measured water solubility – 1.75 mg/L at 25 degrees C and measured VP – 0.0727 Pa at 25 degrees C, SMILES structure input.
Year	2003
Media	Air-Water Soil-Sediment-Partition Coefficient
Estimated Distribution and Media Concentration Model data and results	Air = 0.188%, Water = 5.58%, Soil = 38.6% and Sediment = 55.6%
Remarks	
Data Qualities Reliabilities	Reliability 4. Not assignable.
Remarks for Data Reliability	The data are obtained by a recognized fugacity calculation method. However, the method is an estimation.
References	USEPA, 2003 SRC EPIWIN Program

3 Ecotoxicity

3.1 Acute Toxicity to Fish

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Purity 99.15% isomeric mixture
Method/guideline	OECD 204
Test Type	96-hour acute toxicity to the bluegill sunfish
GLP	Yes
Year	1996
Species/Strain/Supplier	Lepomis macrochirus
Exposure Period	21 days
Analytical monitoring	For the determinations if the test article concentrations, samples of all concentrations and of the control with solubilizers were taken in quadruplicate at the beginning (day 0), day 9 and on day 21 of the test. Samples were analyzed by HPLC.
Remarks for Test Conditions	Flow-through conditions. Nominal concentrations were 0.125 to 2.0 step size. Ten fish were tested at each concentration and the untreated controls. Tests were not conducted in replicate. The average biomass at the start of the test was 0.12 – 0.16 g fish/liter test medium. The oxygen concentration was measured to be at saturation ranging from 6.6 to 9.3 mg O2 per liter and the pH ranged from 7.9 to 8.2. The water temperature throughout the entire test ranged from 19 – 22 degrees C. Total hardness was reported to be 41.2 fr.H°.
Endpoint value	LC50 = 0.452, NOEC = 0.093, LC100=0.83, LOEC= 0.182
Unit	mg/L
Results	Results are expressed based on the mean measured concentrations of HHCB in the test which were 0.093, 0.182, 0.393, 0.830 and 1.566 mg/L. After 21 days of exposure, no effect on fish growth and mortality were observed at the lowest test concentration of 0.093 mg/L. At the next higher concentration of 0.182 mg/L, 10% mortality and no effect on fish growth were observed. At 0.393 mg/L, 10% mortality and a reduced weight and size gain were observed when evaluated by the Dunnett test and in comparison to the control group. At 0.830 and 1.566 mg/L, 100% mortality was observed after 14 and 6 days of exposure, respectively. No clinical signs were observed over the 21-day period in the 0.093 mg/L test concentration. At 0182, 0.393, 0.830 and 1.566 mg/L, clinical

	signs included loss of equilibrium and righting reflex, irregular respiration, and bottom and tail dominant swimming were observed in a significant number of fish. These symptoms were dose dependent with respect to onset and severity.
Conclusion Remarks	
Data Qualities Reliabilities	Reliability 1. Reliable without restriction
Remarks for Data Reliability	Study conducted according to an OECD protocol under GLP and the data are published in a peer-reviewed journal.
Reference	Balk F and Ford RA, 1999b. Environmental risk assessment for the polycyclic musks AHTN and HHCB in the EU. II. Effect Assessment and Risk Characterization. Toxicology Letters, 111, 81-94. Wuthrich, V. 1996a. HHCB: 21-day prolonged toxicity study in the bluegill sunfish under flow-through conditions. Report to RIFM., RCC Umweltchemie AG Project 380711.

3.2 Acute Toxicity to Aquatic Invertebrates

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Purity 99.15% isomeric mixture
Method/guideline	OECD 202
Test Type	Semi-static 21-day Daphnia
GLP	Yes
Year	1996
Species/Strain/Supplier	Daphnia magna
Analytical procedures	Test concentrations were analyzed by HPLC analyses. 48-hour LC50 was calculated based on the 21-day test.
Test Details	Nominal concentrations ranged from 0.062 to 1.0 mg/l. Step size 2. The Logit model (Cox, D.R. Analysis of binary data 1977) was used to compute the EC50 value.
Remarks for Test Conditions	The water chemistry parameters measured were pH, dissolved oxygen, and temperature. The pH of the untreated test medium with and without solubilizers, and the 0.049 and 0.842 mg/L test solutions measured at the beginning and at the end of the respective exposure periods ranged from 7.5 to 8.6 and the DO for the same solutions ranged from 5.3 to 9.0 mg oxygen/L. The room temperature was monitored continuously

	and plotted throughout the entire test. The temperature ranged from 19 to 22 degrees C.
EC50, EL50, LC0, at 24,48 hours	NOEC(rep) = 0.111, LOEC = 0.205, EC50 = 0.282 (48-hrs)
Unit	mg/L
Biological observations	10 daphnids were exposed to each test concentration. The cumulative mortality is tabulated below.
Remarks for Results	Results are based on the measured concentrations of HHCB in the test which were 0.049, 0.111, 0.205, 0.419, and 0.842 mg/L. 48-hour LC_{50} was calculated based on the 21-day test.
Data Qualities Reliabilities	Reliability 1. Reliability without restriction
Data Reliability Remarks	Study was conducted according to an OECD protocol under GLP and the data are published in a peer-reviewed journal.
Reference	Balk F and Ford RA, 1999b. Environmental risk assessment for the polycyclic musks AHTN and HHCB in the EU. II. Effect Assessment and Risk Characterization. Toxicology Letters, 111, 81-94. Wuthrich, V. 1996b. Influence of HHCB on the reproduction of Daphnia magna. Report to RIFM. RCC Umweltchemie AG Project 380687.

Survival of parent daphnids exposed to various concentrations of HHCB in a semi-static system for 21 days

Exposure Day	Nominal and Mean Measured Concentrations of Test Article (mg/L)						
	Control + Solubilizers	Control	0.062	0.125	0.250	0.500	1
	Control + Solubilizers	Control	0.049	0.111	0.205	0.419	0.842
0	10	10	10	10	10	10	10
3	10	10	10	10	10	10	6
6	10	10	10	10	10	8	0
8	10	10	10	10	10	6	0
10	10	10	10	10	10	6	0
11	10	10	10	10	10	6	0
12	10	10	10	10	10	6	0
13	10	10	10	10	10	1	0
14	10	10	10	9	10	1	0
15	10	10	10	9	10	1	0
16	10	10	10	9	10	1	0
17	10	10	10	9	10	0	0
18	10	10	10	9	10	0	0
19	10	10	10	9	10	0	0
20	10	10	10	9	10	0	0
21	10	10	10	9	10	0	0
% Survival	100	100	100	90	100	0	0

3.3 Acute Toxicity to Aquatic Plants

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Purity 99.15% isomeric mixture
Method/guideline	OECD 201
Test Type	Algae Static growth inhibition test
Species/Strain/Supplier	Pseudokirchneriella subcapitata
Exposure Period	72 hours
Remarks for Test Conditions	The test solutions and the untreated test medium were incubated in triplicate and the solvent control was incubated in six replicates of 50 ml. Endpoint was growth rate as well as biomass. Per dose group, an initial algal population of 10,000 cells per ml of test solution was exposed to the test article concentrations or untreated test medium with solvent and without. Test concentrations were determined by HPLC analysis. The pH for all test concentrations was 8.1 at the start of the test and ranged from 9 -10 at the end of the test. The pH of the controls was 8.1 at the start of the test and increased to 10.0 and 9.8 in the solvent control and the untreated control, respectively, by the end of the test. This increase slightly exceeds the tolerance of 1.5 pH units given by the guidelines, but can be justified by the high cell density in the controls after 72 hours of exposure which results in an increased CO2 consumption. The rapid growth resulted in a pH increase of > 1.5 units, although the solutions were continuously stirred at 500 rpm. The hardness of the test medium corresponded to about 24 mg CaCO3/L. The test was conducted at 24 degrees C under continuous illumination.
Results	Nominal concentrations ranged from 0.065 to 1.0 mg/L. Start concentrations were 71-102% of nominal and end concentrations 54-85% of nominal. Mean measured concentrations were 0.042, 0.084, 0.201, 0.466 and 0.854 mg/L. Results are based on the mean measured concentrations. Cells were not removed prior to measurement. After 24, 48 and 72 hours of exposure, the cell density in the test solutions was determined and compared to the cell density in the solvent control. With respect to the biomass, in the solvent control, no significant inhibition of the algae growth was found at the measured test concentrations from 0.084 to 0.201 mg/L. The inhibition of the algae growth at the lowest concentration measured (0.042 mg/L) was on the border of being insignificant. At the highest measured concentrations of 0.466 and 0.854 mg/L significant inhibition was observed. With respect to the growth rate in the solvent control, negligible inhibition was observed from 0.042 to 0.201 mg/L and significant inhibition was observed at 0.466 and 0.854 mg/L.

Endpoint value	NOEC = 0.201, LOEC = 0.466, EC50 for biomass production = 0.72 mg/L, EC50 for growth = > 0.854 mg/L.
Conclusion Remarks	
Data Qualities Reliabilities	Reliability 1. Reliability without restriction
Remarks for Data Reliability	Study conducted according to an OECD protocol under GLP and the data are published in a peer-reviewed journal.
Reference	Balk F and Ford RA, 1999b. Environmental risk assessment for the polycyclic musks AHTN and HHCB in the EU. II. Effect Assessment and Risk Characterization. Toxicology Letters, 111 81-94. Van Dijk, A. 1997. Acute toxicity of HHCB to Pseudokirchneriella subcapitata. Report to RIFM, RCC Umweltchemie AG Project 380632.

4 Human Health Toxicity

4.1 Acute Toxicity

Substance Name	HHCB
CAS No.	1222-05-5
Purity of the Material	The HHCB tested was a commercial sample which is approximately a 65% solution of HHCB in a diluent (diethyl phthalate or isopropyl myristate) to facilitate handling.
Method/guideline	
Test Type	Acute oral toxicity limit test
GLP	Pre-GLP
Year	1975
Species/strain	Rats, Wistar
Sex	Male and Female
# of animals per sex per dose	10
Vehicle	None
Route of Administration	Gavage
Remarks for Test Conditions	14-day observation period
Value LD50 or LC50 with confidence limits	> 3.25 g/kg
Number of deaths at each dose level	1/10 at 3.25 g/kg
Remarks for Results	The material as tested was a commercial sample and therefore, would have been an approximately 65% solution. Therefore the dose administered has been corrected from 5g/kg to 3.25 g/kg bw. No information is present on whether body weight measurements were taken and gross necropsy performed.
Data Qualities Reliabilities	Reliability code 2. Reliable with restrictions
Remarks for Data Reliability	Data collected prior to GLP by method comparable to present guidelines/standards.
References	Moreno, O.M. 1975. Galaxolide 50: acute oral toxicity in rats; dermal toxicity in rabbits. Project No. MB 75-770. MB Research Report to the Research Institute for Fragrance Materials, Inc. (RIFM). Ford RA, 1998. The human safety of the polycyclic musks, AHTN and HHCB in fragrances – A review, Dtsch, Lebens. Rdsch., 98(8), 268-275.

Substance Name	HHCB
CAS No.	1222-05-5
Method/guideline	
Test Type	Acute oral toxicity limit test
Purity of Test Material	The HHCB tested was a commercial sample which is approximately a 65% solution of HHCB in a diluent (diethyl phthalate or isopropyl myristate) to facilitate handling.
GLP	Pre-GLP
Year	1977
Species/strain	Rats
Sex	Female
# of animals per sex per dose	10
Vehicle	None
Route of Administration	Gavage
Remarks for Test Conditions	14-day observation period
Value LD50 or LC50 with confidence limits	> 3 g/kg
Number of deaths at each dose level	0 at highest dose
Remarks for Results	The material as tested was a commercial sample and therefore, would have been an approximately 65% solution. No information is present on whether body weight measurements were taken and gross necropsy performed.
Data Qualities Reliabilities	Reliability code 2. Reliable with restrictions
Remarks for Data Reliability	Data collected prior to GLP by method comparable to present guidelines/standards.
References	Ford RA, 1998. The human safety of the polycyclic musks, AHTN and HHCB in fragrances – A review, Dtsch, Lebens. Rdsch., 98(8), 268-275.

Substance Name	HHCB
CAS No.	1222-05-5
Purity of Test Material	The HHCB tested was a commercial sample which is approximately a 65% solution of HHCB in a diluent (diethyl phthalate or isopropyl myristate) to facilitate handling.
Method/guideline	
Test Type	Acute dermal toxicity limit test
GLP	Pre-GLP
Year	1975
Species/strain	New Zealand white rabbits
Sex	Not reported
# of animals per sex per dose	7
Vehicle	None
Route of Administration	Dermal
Remarks for Test Conditions	14-day observation period
Value LD50 or LC50 with confidence limits	> 3.25 g/kg
Number of deaths at each dose level	0/10 at 3.25 g/kg
Remarks for Results	The material tested was a commercial sample and therefore, would have been an approximately 65% solution. Therefore, the dose administered has been corrected from 5g/kg to 3.25 g/kg bw. There were no deaths at that dose. Therefore, the LD50 can be listed as >3.25 g/kg bw. No information is present on whether body weight measurements were taken and gross necropsy performed.
Data Qualities Reliabilities	Reliability code 2. Reliable with restrictions
Remarks for Data Reliability	Data collected prior to GLP by method comparable to present guidelines/standards.
References	Moreno, O.M. 1975. Galaxolide 50: acute oral toxicity in rats; dermal toxicity in rabbits. Project No. MB 75-770. MB Research Report to the Research Institute for Fragrance Materials, Inc. (RIFM). Ford RA, 1998. The human safety of the polycyclic musks, AHTN and HHCB in fragrances – A review, Dtsch, Lebens. Rdsch., 98(8), 268-275.

Substance Name	HHCB
CAS No.	1222-05-5
Purity of Test Material	The HHCB tested was a commercial sample which is approximately a 65% solution of HHCB in a diluent (diethyl phthalate or isopropyl myristate) to facilitate handling.
Method/guideline	
Test Type	Acute dermal toxicity limit test
GLP	Pre-GLP
Year	1977
Species/strain	CRL Sprague-Dawley
Sex	Female
# of animals per sex per dose	5
Vehicle	Ethanol
Route of Administration	Dermal
Remarks for Test Conditions	7-day observation period
Value LD50 or LC50 with confidence limits	> 5 g/kg
Number of deaths at each dose level	0/5 at 5 g/kg
Remarks for Results	No information is present on whether body weight measurements were taken and gross necropsy performed.
Data Qualities Reliabilities	Reliability code 2. Reliable with restrictions
Remarks for Data Reliability	Data collected prior to GLP by method comparable to present guidelines/standards.
References	Ford RA, 1998. The human safety of the polycyclic musks, AHTN and HHCB in fragrances – A review, Dtsch, Lebens. Rdsch., 98(8), 268-275.

4.2 Genetic Toxicity

4.2.1 In vitro Genotoxicity

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Colorless viscous liquid sample supplied by IFF. Purity > 99% based on isomeric mixture.
Method/guideline	OECD 471
Test Type	Ames reverse mutation assay
System of Testing	
GLP	Yes
Year	1999
Species/Strain	Salmonella typhimurium TA98, TA100, TA1535, TA1537 and TA1538; Escherichia coli WP2 uvrA
Metabolic Activation	With and without S9 activation
Doses/Concentration	10, 33, 100, 333, 1000 or 5000 ug per plate
Statistical Methods	
Remarks for Test Conditions	All positive controls gave positive responses to the systems within acceptable ranges.
Results	No significant increase in the number of revertant colonies was observed with HHCB at doses of 10-5000 ug/plate.
Cytotoxic concentration	None
Genotoxic Effects	None
Conclusion Remarks	No mutagenic potential
Data Qualities Reliabilities	Reliability 1. Reliable without restrictions.
Remarks for Data Reliability	Data are by a standard method and have been published in a peer-reviewed journal.
References	Api, AM and San, RHC, 1999. Genotoxicity Tests with 6-Acetyl-1,1,2,4,4,7-hexamethyltetraline and 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-gamma-benzopyran. Mutation Research, 446: 67-81.

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Material was obtained from a commercial source, Promochem, under the trade name Galaxolide. Galaxolide is sold as a 65% solution in diethyl phthalate.
Method/guideline	Not reported
Test Type	Ames reverse mutation assay
System of Testing	
GLP	Not reported
Year	1998
Species/Strain	Salmonella typhimurium TA97, TA98, TA100 and TA102
Metabolic Activation	With and without S9 activation
Doses/Concentration	5-500 ug per plate (corrected concentrations range from 3.25 to 325 ug/plate based on testing of 65% Galaxolide)
Statistical Methods	
Remarks for Test Conditions	The doses were 5, 16.6, 50, 166.6 or 500 ug/plate (limit of solubility)
Results	No significant increase in the number of revertant colonies was observed with HHCB at doses of 5-500 ug/plate.
Cytotoxic concentration	None
Genotoxic Effects	None
Conclusion Remarks	No mutagenic potential
Data Qualities Reliabilities	Reliability 1. Reliable without restrictions.
Remarks for Data Reliability	Data are by a standard method and have been published in a peer-reviewed journal.
References	Mersch-Sundermann V, Kevekordes S and Jenter C, 1998a. Lack of mutagenicity of polycyclic musk fragrances in Salmonella typhimurium. Tox. In Vitro, 12: 389-393.

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Material was obtained from a commercial source, Promochem, under the trade name Galaxolide. Galaxolide is sold as a 65% solution in diethyl phthalate.
Method/guideline	Not reported
Test Type	In vitro micronucleus
System of Testing	
GLP	No
Year	1997
Species/Strain	Human peripheral lymphocytes from healthy non-smoking donors
Metabolic Activation	With and without S9 activation
Doses/Concentration	0.05, 0.49, 4.85, 48.5, 97 micromolar. (corrected for 65% solution = 0.0325, 0.3185, 3.152, 31.52, 63.05, 126.1 uM)
Statistical Methods	
Remarks for Test Conditions	Positive controls significantly increased the frequency of micronuclei.
Results	No significant increase in the frequency of micronuclei
Cytotoxic concentration	194 micromolar
Genotoxic Effects	None
Conclusion Remarks	No mutagenic potential
Data Qualities Reliabilities	Reliability 1. Reliable without restrictions.
Remarks for Data Reliability	Data are by a standard method and have been published in a peer-reviewed journal.
References	Kevekordes S, Mersch-Sundermann V, Diez M and Dunkelberg H, 1997. In vitro genotoxicity of polycyclic musk fragrances in the micronucleus test. Mutation Research, 395 (2-3), 145-150.

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Material was obtained from a commercial source, Promochem, under the trade name Galaxolide. Galaxolide is sold as a 65% solution in diethyl phthalate.
Method/guideline	Not reported
Test Type	In vitro micronucleus
System of Testing	
GLP	No
Year	1997
Species/Strain	Human hepatoma cells
Metabolic Activation	With and without S9 activation
Doses/Concentration	0.1, 0.97, 9.7, 97, 194, 387 micromolar (corrected for 65% solution = 0.065, 0.6305, 6.305, 63.05, 126.1, 251.5 uM)
Statistical Methods	
Remarks for Test Conditions	Incubation period was 2 hours after which, the cells were harvested and scored for micronuclei.
Results	No significant increase in the frequency of micronuclei was seen with HHCB treatment.
Cytotoxic concentration	387 micromolar
Genotoxic Effects	None
Conclusion Remarks	No mutagenic potential
Data Qualities Reliabilities	Reliability 1. Reliable without restrictions.
Remarks for Data Reliability	Data are by a standard method and have been published in a peer-reviewed journal.
References	Kevekordes S, Mersch-Sundermann V, Diez M and Dunkelberg H, 1997. In vitro genotoxicity of polycyclic musk fragrances in the micronucleus test. Mutation Research, 395 (2-3), 145-150.

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Colorless viscous liquid sample supplied by IFF. Purity > 99% based on isomeric mixture.
Method/guideline	OECD 482
Test Type	In vitro unscheduled DNA synthesis
System of Testing	
GLP	Yes
Year	1999
Species/Strain	Primary rat hepatocytes from Sprague-Dawley rats
Metabolic Activation	With and without S9 activation
Doses/Concentration	0.15, 0.50, 1.5, 5, 15, 50 ug/ml
Statistical Methods	
Remarks for Test Conditions	Positive control induced a significant increase in the average net nuclear grain count over controls.
Results	No significant increase in UDS
Cytotoxic concentration	50 ug/ml
Genotoxic Effects	None
Conclusion Remarks	No genotoxic potential
Data Qualities Reliabilities	Reliability 1. Reliable without restrictions.
Remarks for Data Reliability	Data are by a standard method and have been published in a peer-reviewed journal.
References	Api, AM and San, RHC, 1999. Genotoxicity Tests with 6-Acetyl-1,1,2,4,4,7-hexamethyltetraline and 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-gamma-benzopyran. Mutation Research, 446: 67-81.

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Material was obtained from a commercial source, Promochem, under the trade name Galaxolide. Galaxolide is sold as a 65% solution in diethyl phthalate.
Method/guideline	Not reported but similar to OECD 479
Test Type	Sister-chromatid exchange (SCE)
System of Testing	
GLP	Not reported
Year	1998
Species/Strain	Human lymphocytes obtained from healthy non-smoking donors
Metabolic Activation	With and without S9 activation
Doses/Concentration	0.025, 0.25, 2.43, 24.25, 48.5 or 97 micromolar (corrected for 65% solution = 0.0162, 0.1625, 1.579, 15.76, 31.52, 63.05 uM)
Statistical Methods	
Remarks for Test Conditions	Treatment time was 2 hours. Positive controls showed a significant increase in SCEs.
Results	No significant increase in the number of sister chromatid exchanges was observed with HHCB at the doses tested compared to non-treated lymphocytes.
Cytotoxic concentration	97 micromole
Genotoxic Effects	None
Conclusion Remarks	No mutagenic potential
Data Qualities Reliabilities	Reliability 1. Reliable without restrictions.
Remarks for Data Reliability	Data are by a standard method and have been published in a peer-reviewed journal.
References	Kevekordes S, Mersch-Sundermann V, Diez M, Bolten C and Dunkelberg H, 1998. Genotoxicity of polycyclic musk fragrances in the Sister-Chromatid Exchange test. Anticancer Research,

18: 449-452.

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Colorless viscous liquid sample supplied by IFF. Purity > 99% based on isomeric mixture.
Method/guideline	OECD 473
Test Type	Chormosome aberration with multiple harvest times
System of Testing	
GLP	Yes
Year	1999
Species/Strain	Chinese hamster ovary cells
Metabolic Activation	With and without S9 activation
Doses/Concentration	Wo/activation for 4/20, 20/20, 44/44 hr exposure/harvest (e/h) times at 5, 10, 20 microgram/ml; w/activation for 4/20 hr e/h with 9, 17, 34 microgram/ml and for 4/44 hr e/h with 23, 28, 30 microgram/ml
Statistical Methods	
Remarks for Test Conditions	Cells were assessed for structural chromosome aberrations at the 20 and 44-hr harvest times. Numerical chromosome aberrations were also assessed at the 44-hr harvest. MNNG was used as the positive control in the non-activated study at a final concentration of 2 ug/ml. B(a)P was used as the positive control in the S-9 activated study at a final concentration of 30 ug/ml. Statistical analysis of the percent aberrant cells was performed using the Fisher's exact test. The Fisher's test was used to compare pair-wise the percent aberrant cells of each treatment group with that of the solvent control. A positive Fischer's test at any dose level is followed by the Cochran-Armitage test which is used to measure dose-responsiveness. The test article was considered to induce a positive response when the percentage of cells with aberrations increased in a dose-response manner with one or more concentrations being statistically elevated relative to the solvent control group. A reproducible and statistically significant increase at a single

	dose was considered positive. The test was determined to be valid, as a statistically significant increase in the percentage of cells with chromosome aberrations were observed in the positive control relative to the solvent control.
Results	A minimum of 200 metaphase spreads were examined for each concentration and scored for chromatid–type and chromosome-type aberrations. No significant increase in structural or numerical chromosome aberrations
Cytotoxic concentration	20 ug/ml w/o activation; 30 ug/ml with activation
Genotoxic Effects	None
Conclusion Remarks	No genotoxic potential
Data Qualities Reliabilities	Reliability 1. Reliable without restrictions.
Remarks for Data Reliability	Data are by a standard method and have been published in a peer-reviewed journal.
References	Api, AM and San, RHC, 1999. Genotoxicity Tests with 6-Acetyl-1,1,2,4,4,7-hexamethyltetraline and 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-gamma-benzopyran. Mutation Research, 446: 67-81.

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Material was obtained from a commercial source, Promochem, under the trade name Galaxolide. Galaxolide is sold as a 65% solution in diethyl phthalate.
Method/guideline	Not reported
Test Type	In vitro
System of Testing	SOS induction using E. coli PQ37
GLP	No
Year	1998
Species/Strain	Human hepatoma cells
Metabolic Activation	With and without S9 activation
Doses/Concentration	0.39, 0.78, 1.56, 3.125, 6.25, 12.5, 25 or 50 ug (corrected for 65% solution = 0.25, 0.507, 1.014, 2.03, 4.06, 8.12, 16.25,

	and 32.5 ug)
Statistical Methods	
Remarks for Test Conditions	Following a 2-hour incubation period, beta-galactosidase and alkaline phosphotase activities were measured.
Results	Both positive controls significantly increased inducing factors (IF) but no induction activity or toxicity was seen with HHCB at any dose.
Cytotoxic concentration	
Genotoxic Effects	None
Conclusion Remarks	No mutagenic potential
Data Qualities Reliabilities	Reliability 1. Reliable without restrictions.
Remarks for Data Reliability	Data are by a standard method and have been published in a peer-reviewed journal.
References	Mersch-Sundermann V, Kevelordes, S., and Jenter, C. 1998b. Testing of SOS induction of Artificial Polycyclic Musk Fragrances in E. coli PQ37. Toxicology Letters, 95: 147-154.

4.2.2 In vivo Genotoxicity

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Colorless viscous liquid sample > 99% purity based on isomeric mixture.
Method/guideline	OECD 474
Test Type	In Vivo mouse micronucleus cytogenetic assay
GLP	Yes
Year	1999
Species/Strain	ICR mice
Sex	Male and Female
Route of Administration	Intraperitoneal
Doses/Concentration	376, 750 or 1500 mg/kg
Exposure Period	Bone marrow cells were harvested and evaluated 24, 48 and 72 hours after dosing.

Remarks for Test Conditions	Negative control was corn oil and positive control was cyclophosphamide
Appropriate statistical evaluations?	
Effect on mitotic index or PCE/NCE ratio by dose level and sex	Moderate reductions (up to 25%) in the ratio of PCE to total erythrocytes were observed in groups on 1500 mg/kg bw after 48 and 72 hours indicating toxicity and bioavailability to the bone marrow.
Genotoxic effects	None
NOEL (C)/ LOEL (C)	
Remarks for Results	
Conclusion Remarks	No significant increases in micronucleated PCE in any of the HHCB-treated groups relative to the respective vehicle control group were observed in male or female mice at 24, 48 or 72 hours after dose administration.
Data Qualities Reliabilities	Reliability 1. Reliable without restrictions.
Remarks for Data Reliability	Data are by a standard method and have been published in a peer reviewed journal.
References	Api, AM and San, RHC, 1999. Genotoxicity Tests with 6-Acetyl-1,1,2,4,4,7-hexamethyltetraline and 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-gamma-benzopyran. Mutation Research, 446: 67-81.

4.3 Repeat dose Toxicity

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Colorless viscous liquid sample > 99% purity based on isomeric mixture.
Method/guideline	OECD 408
GLP	Yes
Year	1999
Species/strain	Crl:CD(SD)Br
Sex	Male and Female
Route of Administration	Diet
Doses/concentration Levels	5, 15, 50 or 150 mg/kg per day; 15M and 15F per dose
Exposure Period	13 weeks

Frequency of Treatment	Daily
Control Group	Diet only
Post Exposure	4 weeks post exposure observation were conducted on selected rats from control and high dose groups
Remarks for Test Conditions	A two-week range finding study was conducted at nominal doses of 0, 300, 600 or 1000 mg HHCB/kg/ per day. At the end of the 14-day period, all animals were sacrificed and examined macroscopically and the livers and kidneys were weighed and examined microscopically. A progressive dose-related decrease in body weight gain was seen at the 3 higher doses. A dose-related increase in liver weights at all doses and moderate centrilobular hypertrophy in the liver in 1/5 males and 2/5 females at the higher doses was observed. Since a NOEL was not observed in the range finding study, the LOAEL was established at 300 mg/kg/day. Nominal doses of 5, 15, 50 and 150 mg/kg per day were selected for the 90-day study. HHCB was added to the diet to the desired concentration. The mean achieved daily intakes were 5.4, 15.7, 51.8 and 155.8 mg HHCB/kg bw for males and 5.1, 15.6, 51.9 and 154.6 mg HHCB/kg bw for females.
NOAEL (NOEL)	150 mg/kg based on final study
Toxic Response/effects by Dose Level	LOAEL based on 2 week range finding study = 300 mg/kg (increased liver weights seen at this dose). On completion of the treatment or treatment-free periods in the main study, all animals were sacrificed and examined macroscopically. Organs were weighed and tissues preserved for histopathological examination. Histopathological examinations were also conducted on the male and female reproductive organs of all animals in all dose groups. There were no mortalities or adverse clinical signs. Body weight gain and food consumption were increased in males, but not females, at all doses. This increase was not statistically significant and no correlation with dose was seen. No changes in hematology or ophthalmologic evaluation were observed. At weeks 7 and 13, slightly lower mean plasma triglyceride levels were observed for males given 15, 50 and 150 mg/kg per day. However, all mean values, except for the two highest dosed male groups at week 13, were within the standard deviation of the controls. A slightly lower plasma glucose concentration was noted at week 7 in males and females given 15, 50 and 150 mg/kg per day and week 13 in males given 50 and 150 mg/kg/day. None of these differences between control and treated groups were present at the end of the treatment-free period. There were no statistically significant differences in absolute or bodyweight related organ weights after 13 weeks of treatment. However, when liver weight was compared with brain weight, statistically significant higher values were seen in all dosed males compared to control males. The liver to brain weight differences correspond to the increased body weight gains of the dosed groups and are therefore, not considered an adverse effect. There was no evidence of a dose response relationship

	and also no correlation to any histopathological changes in the liver or brain. Histopathological examination of the ovaries and vaginas showed an association between the distension and the proestrus stage of the life cycle. No other histopathological effects in male or female reproductive organs were found in any dose group. No abnormalities of any tissues were observed at necropsy or in histopathology. Since there were no significant adverse effects at any dose level, the NOAEL was determined to be > 150 mg/kg.
Statistical Evaluation	
Conclusion Remarks	
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Study exceeded requirements of OECD 408 and are published in a peer reviewed journal.
References	Api, AM and Ford, RA, 1999. Evaluation of the Oral Subchronic Toxicity of HHCB (1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-gamma-2-benzopyran) in the Rat. Toxicology Letters, 111: 143-149.

4.4 Reproductive Toxicity

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Viscous neat material, purity > 95% supplied by IFF.
Method/guideline	There are no specific regulatory guidelines for this study although the design took into consideration the principles of the International Conference on Harmonization (ICH) Guidelines on Detection of Toxicity to Reproduction for Medicinal Products endorsed by the ICH Steering Committee Step 4 of the ICH process. 24 June, 1993. Section 4.1.2. This guideline has also been issued by the JMOHW (Japanese Ministry of Health and Welfare): Yakushin No. 470 of 7 July 1994.
Test Type	The primary intention of the study was to investigate whether exposure of the newborn to the test material via maternal milk resulted in any changes in post natal development. The rat was used since this is a universally accepted species in reproductive studies and the laboratory had background control data on reproductive performance, fertility and survival indices in the strain used. The oral route was selected as this is a potential route of human exposure with possible exposure of the fetus in utero and of the neonate via maternal milk.
GLP	Yes
Year	1996

Species/Strain	Crl:CD(SD)Br
Sex	Female and Male (only F0 females were dosed)
Route of Administration	Oral gavage in corn oil
Duration of Test	19 weeks
Doses/Concentration	The doses administered were 2, 6 and 20 mg/kg/day. Dosages were selected based upon the results of a milk transfer study performed in these laboratories in which a dosage of 20 mg/kg/day was associated with maximum HHCB levels in the milk in the range of 2.52 to 17.29 ppm (Day 3 or 7 post partum, 8 hours post dose) and a dosage of 2 mg/kg/day resulted in achieved milk levels in the range of 0.00 to 0.32 ppm (Day 3 or 7 post partum, 4 hours post dose). Treatment of females commenced on Day 14 of pregnancy since it was considered that steady state plasma drug levels would have been achieved by parturition. The dosage volume was calculated for individual females on Days 14 and 16 of pregnancy according to body weight. The F1 offspring were only exposed *in utero* and through mothers' milk from birth to weaning.
Control Group and Treatment	The control group received the vehicle only.
Frequency of Treatment	Daily
Remarks for Test Conditions	A total of 121 sexually mature female rats approximately 8-10 weeks, which were time-mated to males of the same strain, were received at the laboratory from Charles River, UK Ltd., Kent, UK. The first group (A) consisted of 69 animals followed by a second group (B) of 52 animals mated one day later. The day of mating, as judged by the appearance of sperm in the vaginal smear was considered to be Day 0 of pregnancy. An additional batch of 10 animals was supplied with batch A for health check purposes. On arrival, all animals were examined for abnormalities and signs of overt ill health. Those designated as health check animals were killed within 24 hours after arrival and subjected to routine macroscopic examination that revealed no changes associated with infectious disease. F0 animals were re-housed in individual breeding cages for the birth and rearing of the young. During the pre-mating period, F1 generation males and females were housed separately. During the mating period, F1 animals were housed on the basis of one male to one female in plastic breeding cages.
NOAEL(NOEL)	A NOAEL of 20 mg/kg/day was established.
Appropriate statistical evaluations	Significance tests, employing analysis of variance followed by an intergroup comparison with the control, were performed on the following parameters: food consumption, bodyweight change, litter data, sex ratios, pre-weaning development, post weaning behavioral tests and sexual maturation. Dependent on the heterogeneity of variance between treatment groups, parametric tests, followed by a William's test or a non-parametric test followed by Shirley's test were used to analyze

	the data as appropriate. For litter data and pre-weaning development, the basic sampling unit was the litter and due to the preponderance of non-normal distributions non-parametric analyses were routinely used. Where 75% or more of the values for a given variable were the same, a Fisher's exact test was used.
Parental data and F1 as Appropriate	**No effects were seen on F0 females.** The F0 animals were observed for the following: clinical signs, food consumption, bodyweight changes, duration of pregnancy, and litter data (number of pups, sex, abnormalities, non-pregnant females were sacrificed and their uteri examined for evidence of implantation.). The F1 generation pups were evaluated for surface righting reflex, startle reflex, air righting reflex and pupil reflex. At the end of the pre-weaning period, 24 male and 24 female pups per group were retained for further study. On Day 22 post partum excess pups and parents were sacrificed and examined externally and internally for abnormalities. **No effects were seen on F1 males or females.** The FI generation was not dosed directly but were exposed via mothers milk. The animals were regularly handled and observed for overtly abnormal behavior and any obvious changes. Animals were also weighed weekly. In addition, they were examined using 1) the accelerating rotarod test, 2) Actimat test, and 3) one trial passive avoidance test. When the offspring were approximately 84 days of age, they were mated on a one male to one female basis for 20 days. Brother and sister pairings were avoided. Dams that litter were weighed on Days 0, 7, 14, and 21 post partum. As soon as possible after parturition, the young (F2 generation) within each littler were counted, individually identified and examined for abnormalities. On or shortly after Day 21 post partum, all F2 pups and F1 dams were sacrificed and examined internally and externally for abnormalities.
Remarks for Results	Exposure to test material by gavage to F0 dams had no effects at any dose and exposure to F1 offspring through mother's milk had no effects on behavior or reproductive performance. F2 pups were without adverse effects. Under the conditions of this study, the NOAEL on the pregnant and lactating rat and peri and post natal development of the offspring was considered to be 20 mg/kg/day.
Data Reliabilities Qualities	Reliability code 1. Reliable without restrictions.
Remarks for Data Reliability	Study was conducted according to a recognized guideline and under GLP.
References	Ford, RA and Bottomley, A, 1997. A Method for Evaluation of the Potential Toxicity to the Neonate from Exposure to Xenobiotics via Mother's Milk – Application to Three Fragrance materials. The Toxicologist 36, No.1, Part 2:367. Jones, K., Bottomley A.M. and Gopinath, C. (1996) HHCB: Effects on peri- and post natal development including maternal function in the rat (Gavage administration). Report to RIFM. September, 1996

4.5 Developmental/Teratogenicity Toxicity

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Viscous neat material, purity > 95% supplied by IFF.
Test Type	Developmental toxicity. The protocol is cited to be in compliance with OECD Section 4, No.414, Teratogenicity, pp. 1-6.
GLP	Yes
Year	1999
Species/strain	Rat/Crl:CD(SD)Br VAF/Plus (Sprague-Dawley)
Sex	Female
Route of Administration	Oral gavage in corn oil
Duration of Test	3 weeks
Doses/concentration Levels	0, 50, 150 and 500 mg/kg/day
Exposure Period	Days 7 through 17 of pregnancy
Frequency of Treatment	Daily
Control Group and Treatment	Corn oil only
Remarks for Test Conditions	The study was conducted in accordance with ICH Harmonized Tripartite Guideline Stages C and D. 215 healthy virgin female rats were placed into cohabitation for a maximum of 5 days with 215 breeder male rats (one male rat per female rat in the male rat's cage). Female rats with spermatozoa observed in a smear of the vaginal contents were considered to be at Day 0. 25 female, pregnant rats were assigned to each of the four dosage groups 0, 50, 150 and 500 mg/kg. The female rats were observed for viability at least twice each day during the study and for general appearance twice during acclimation on Day 0. Body weights were recorded twice during acclimation. Body weights were recorded on Day 0 and daily during the dosage and postdosage periods. Feed consumption values were recorded on Days 0, 7, 10, 12, 15, 18 and 20. All rats were sacrificed on Day 20 and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. The uterus of each rat was excised and examined for pregnancy, number and distribution of implantations, live and dead fetuses and early and late resorptions. Each fetus was weighed and examined for

	sex and gross external alterations. Approximately one-half of the fetuses in each litter were examined for soft tissue alterations. The remaining fetuses in each litter were eviscerated and examined for skeletal alterations.
NOAEL(NOEL) maternal toxicity	50 mg/kg/day
LOAEL(LOEL) maternal toxicity	150 mg/kg/day
NOAEL (NOEL) developmental toxicity	150 mg/kg/day
LOAEL developmental Toxicity	500 mg/kg/day
Actual dose received by dose level and sex	
Maternal data with dose level	Reduction in maternal bodyweight gain and feed consumption at two highest doses.
Fetal Data with Dose Level	Decreased mean bodyweights with axial skeleton (vertebral/rib) variations were statistically significant in high dose group only.
Appropriate statistical evaluations	
Remarks for Results	No deaths, abortions, or premature deliveries occurred during the study. All rats survived until scheduled sacrifice. The 500 mg/kg/day dosage group had four to nine rats with excess salivation, urine-stained abdominal fur, red or brown substance on the forepaws and alopecia. All necropsy observations were considered unrelated to the test article. Maternal body weight gains were unaffected by the 50 mg/kg/day dosage of the test article. The 150 and 500 mg/kg/day dosage groups had statistically significant dosage-dependent reductions in maternal body weight gains for the entire dosage period. Absolute and relative feed consumption values were unaffected by the 50 mg/kg/day dosage of the test article. Absolute and relative feed consumption values for the entire dosage period were reduced in the 150 and 500 mg/kg/day. Pregnancy occurred in 21 to 25 of the 25 female rats in each dosage group. The 500 mg/kg/day dosage group had significantly reduced fetal body weights as compared with the control group values. This was considered an effect of the test article because it occurred in the high dosage group, was statistically significant and the values were clearly lower than those for any other dosage group. There were no other dose-dependent or statistically significant differences in the fetuses. Fetal alterations were defined as malformations (irreversible changes) and variations (common findings that are reversible). Significant increases in fetal malformations were seen in the 500 mg/kg/day dosage groups and are considered to be associated with administration of the dosage of the test article to the dams. Malformations occurring in fetuses of dams administered dosages less than 500 mg/kg/day were considered unrelated to the test article because the severity of the expression of the malformation was not consistently dosage-dependent and in some fetuses, malformations of

	concern were present with other alterations considered sporadic in nature, because of the absence of an embryologically-based association. All other gross external, soft tissue and skeletal malformations or variations in the fetuses were considered to be unrelated to the test article. Based on these data, the test article was not selectively toxic to development because adverse effects on development occurred only at doses that produced toxic effects (clinical signs) in the dams.
Conclusion Remarks	
Data Qualities Reliabilities	Reliability code 1. Reliable without restrictions.
Remarks for Data Reliability	Study conducted under GLP and is published in a peer-reviewed journal. The regulations of the US FDA, The Japanese Ministry of Health and Welfare and the European Economic Community were used as the basis for the study design and compliance with GLP. There were no significant deviations from the GLP Regulations that affected the quality or integrity of the study.
References	Christian MS, Hoberman AM, Diener, RM, Parker RM and Api, AM (1999). Developmental toxicity study of four fragrances in rats. Toxicology Letters, 111: 169-174.